Determination of the Fatty Acids of Hibiscus Manihot Seed Oil by GC-MS

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Abstract The oil was extracted from Hibiscus manihot seed using Soxhlet extraction method, petroleum ethersoak method..The fatty acid compositions of seed oil were analyzed by GC/MS and Gas Chromatographic conditionwas optimized. The results showed that the content of linoleic acid, oleic acid and palmitoleic acid were 58.36 %, 1.66 % and21.95 % respectively and unsaturated fatty acids were 75.56 %.

Key words GC-MS；fatty acids；Soxhlet extraction method.

Hibiscus Manihot has the most medicinal and health functions in more than 200 okra plants and has a high medicinal value [1]. Hibiscus Manihot seeds are rich in unsaturated fatty acids such as oleic acid, linoleic acid, arachidonic acid and so on. Unsaturated fatty acids have a significant effect on lowering serum cholesterol in high-density lipoproteins, which in turn reduces the incidence of diseases such as hypertension, heart disease, and stroke, at the same time, unsaturated fatty acids play an important role in maintaining the structure and function of biofilms. Currently unsaturated fatty acid products have been widely used in medicine, nutritional supplements, health foods and other fields. Therefore, the determination of unsaturated fatty acids is of great significance for evaluating the medicinal value of Hibiscus Manihot seeds [2]. There are many analytical methods for the determination of unsaturated fatty acids, such as gas chromatography, silver ion high performance liquid chromatography, GC/MS analysis, and UV-visible spectrophotometric thin-layer chromatography, High performance liquid chromatography, underivatized high performance liquid chromatography [3-7], etc., but there are some problems, such as UV-visible spectrophotometry is more troublesome, the analysis time is longer, and high-performance liquid chromatography requires higher instrumentation, etc. The development of gas chromatography-mass spectrometry (GC-MS) technology is rapid, and GC-MS analysis is used to determine the efficiency of unsaturated fatty acids. In this paper, Hibiscus Manihot seed oil was extracted by soxhlet extraction and petroleum ether soaking. The conditions of gas phase and mass spectrometry were optimized to improve the accuracy of GC-MS analysis results and provide reference data for the discussion of nutrition and market development of Hibiscus Manihot seed oil.

1 Experimental section

1.1 Instruments, reagents, and samples

instrument: Agilent Technologies Gas Chromatograph-Mass Spectrometer (US

Hewlett Packard). 80-2B Desktop Centrifuge (Shanghai Anting Scientific Instrument Factory); Soxhlet Extractor,; RE-52C Rotary Evaporator (Shanghai Yarong Shenghua Instrument Factory); HX-200A High-speed Chinese medicine grinder.

Reagents: petroleum ether (60 ~ 90 °C), n-hexane, methanol, potassium hydroxide, anhydrous sodium sulfate (all analytical grade).

Sample: Hibiscus Manihot seed was collected from A northern city, Democratic People's Republic of Korea. The sample was crushed by a high-speed Chinese medicine grinder and sieved (pore size 0.4 mm) for use.

1. 2 Fat oil extraction

Precision weighing 1.1 processed samples 5.00 g into the filter paper tube, then add to the Soxhlet extractor and add 150 m L of petroleum ether, recirculate in a 70 °C water bath for 6 h, the pale yellow transparent extract was obtained, concentrate under reduced pressure, the fat oil yield is 22. 6%.

1. 3 The methyl esterification of fatty acids [8 - 10]

At 1. 2 in the extracted fatty oil, add 8 m L of n-hexane, 0. 5 m L/L potassium hydroxide methanol solution 2 m L, in the 70 °C water bath reflux 20 min, cooling, adding 12 m L distilled water, ultrasonic 5 min, the solution was centrifuged at 3 000 r / min for 10 min.The supernatant was removed, dried over anhydrous sodium sulfate and centrifuged again. The supernatant was subjected to GC/MS analysis.

1. 4 Gas chromatography-mass spectrometry conditions

1. 4. 1 chromatographic conditions

Column: Agilent 19091S-433UI;HP-5ms Ultra Inert; -60 °C—325 °C (350 °C): 30 m x 250 μm x 0.25 μm, (initial value) 80 °C。Pressure 9.3825 psi,Flow rate 1 mL/min,Average line speed 36.966 cm/sec,Residence time 1.3526 min,Flow program open,Flow program 1 mL/min for 0 min,Run time 26 min.

1. 4. 2 Mass spectrometry conditions

The ion source is an electron bombardment source; the ion source temperature is 325 °C; the ionization voltage is 70 eV; the electron multiplier voltage is 1 988 V; the emission current is 34. 6 μA; interface temperature 325 °C; mass range 20 to 500 m/z.

1. 5 experimental methods

Take 1. 3 sample solution 0. 4 μL for experimentation. Retrieving the NIST98 spectral library from the G1701BA ChemStation data processing system. And compared with the fifteen peak index and the standard spectrum of the EPA/NIH mass spectrum atlas respectively, determine the individual chemical components in the sample. Then we use area normalization method for quantitative analysis, obtain the relative percentage of each chemical component in the sample.

2 Results and analysis

The sample was analyzed and identified by a GC/MS analyzer, the total ion chromatograms of Hibiscus Manihot seed fatty acid methyl esters and their chemical constituents are shown in Figure 1.

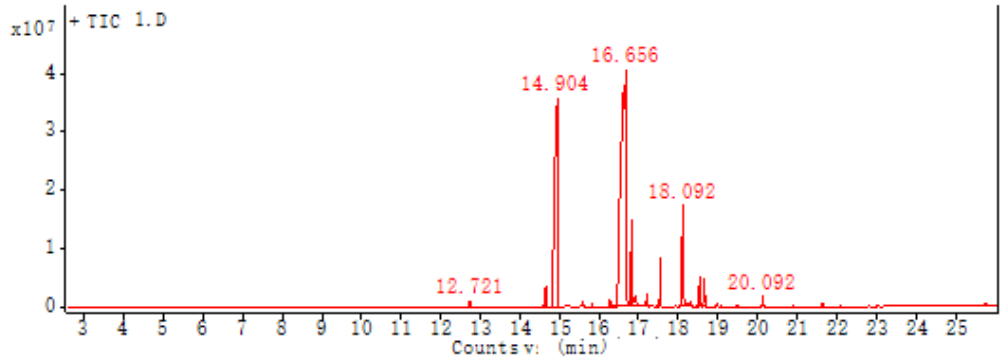

Fig. 1 Total ion chromatogram of fatty acid methyl esters and various chemical components of Hibiscus Manihot seed

The relative percentages of the chemical composition of the fatty acid methyl esters and various chemical components in the sample are given in Table 1,2.

As can be seen from Table 1,2 acorrding keep time,a total of 15 chemical constituents were detected after the methyl esterification of the Hibiscus Manihot seed. In these, chemical constituent for keep time (16.656min) is the highest , next constituents for keep time (14.904min, 18.092 min and 16.774 min) are higher than other. Major chemical constituents are unsaturated fatty acids (Linoleic acid, Linolenic acid)and saturated fatty acids(Hexadecanoic acid, Stearic acid). For them, specific analysis results are given in Fig 2,3,4 and Table 3,4,5 .

Table 1 Chromatogram peak list

No	Keep time	Peak height	Peak height percentage	Area	Area percentage	Area plus percentage	Symmetry factor	Width
1	12.721	1189294.75	2.93	1722256.26	0.43	0.25	1	0.082
2	14.621	3771619.42	9.28	6333136.89	1.59	0.93	1	0.098
3	14.904	35703527.83	87.89	150241570.3	37.61	21.95	0.12	0.201
4	15.568	1094187.08	2.69	2412686.77	0.6	0.35	1.17	0.088
5	16.239	1226132.91	3.02	2618891.51	0.66	0.38	1.73	0.129
6	16.656	40624267.94	100	399446592.9	100	58.36	0.1	0.394
7	16.774	14954868.68	36.81	25292068.04	6.33	3.7	0.52	0.076
8	16.874	2064453.11	5.08	11394464.68	2.85	1.66	1.92	0.212
9	17.156	2378925.52	5.86	4198507.14	1.05	0.61	1	0.076
10	17.509	8438321.53	20.77	13687967.61	3.43	2	0.59	0.082
11	18.092	17361389.44	42.74	41564684.01	10.41	6.07	0.27	0.171
12	18.274	918906.42	2.26	1841931.87	0.46	0.27	0.76	0.124
13	18.503	5299321.33	13.04	12529127.74	3.14	1.83	0.72	0.124
14	18.615	4733603.7	11.65	8540548.94	2.14	1.25	0.69	0.124
15	20.092	1708699.77	4.21	2633804.36	0.66	0.38	0.84	0.095

Table 2 Compound table

No	Compound label	Keep time	name	Molecular formula	MFG Molecular formula	DB Molecular formula
1	Cpd 1: Methyl tetradecanoate	12.721	Methyl tetradecanoate	C15H30O2	C15H30O2	C15H30O2
2	Cpd 2: 9-Hexadecenoic acid, methyl ester, (Z)-	14.621	9-Hexadecenoic acid, methyl ester, (Z)-	C17H32O2	C17H32O2	C17H32O2
3	Cpd 3: Hexadecanoic acid, methyl ester	14.904	Hexadecanoic acid, methyl ester	C17H34O2	C17H34O2	C17H34O2
4	Cpd 4: cis-10-Heptadecenoic acid, methyl ester	15.568	cis-10-Heptadecenoic acid, methyl ester	C18H34O2	C18H34O2	C18H34O2
5	Cpd 5: Methyl 2-octylcyclopropene-1-heptanoate	16.239	Methyl 2-octylcyclopropene-1-heptanoate	C19H34O2	C19H34O2	C19H34O2
6	Cpd 6: 8,11-Octadecadienoic acid, methyl ester	16.656	8,11-Octadecadienoic acid, methyl ester	C19H34O2	C19H34O2	C19H34O2
7	Cpd 7: Methyl stearate	16.774	Methyl stearate	C19H38O2	C19H38O2	C19H38O2
8	Cpd 8: Oleic Acid	16.874	Oleic Acid	C18H34O2	C18H34O2	C18H34O2
9	Cpd 9: Methyl 2-octylcyclopropene-1-octanoate	17.156	Methyl 2-octylcyclopropene-1-octanoate	C20H36O2	C20H36O2	C20H36O2
10	Cpd 10: cis-10-Nonadecenoic acid, methyl ester	17.509	cis-10-Nonadecenoic acid, methyl ester	C20H38O2	C20H38O2	C20H38O2
11	Cpd 11: E,E,Z-1,3,12-Nonadecatriene-5,14-diol	18.092	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	C19H34O2	C19H34O2	C19H34O2
12	Cpd 12: cis-13-Eicosenoic acid, methyl ester	18.274	cis-13-Eicosenoic acid, methyl ester	C21H40O2	C21H40O2	C21H40O2
13	Cpd 13: 6,9,12-Octadecatrienoic acid, methyl ester	18.503	6,9,12-Octadecatrienoic acid, methyl ester	C19H32O2	C19H32O2	C19H32O2
14	Cpd 14: Oxiraneoctanoic acid, 3-octyl-, cis-	18.615	Oxiraneoctanoic acid, 3-octyl-, cis-	C18H34O3	C18H34O3	C18H34O3
15	Cpd 15: Docosanoic acid, methyl ester	20.092	Docosanoic acid, methyl ester	C23H46O2	C23H46O2	C23H46O2

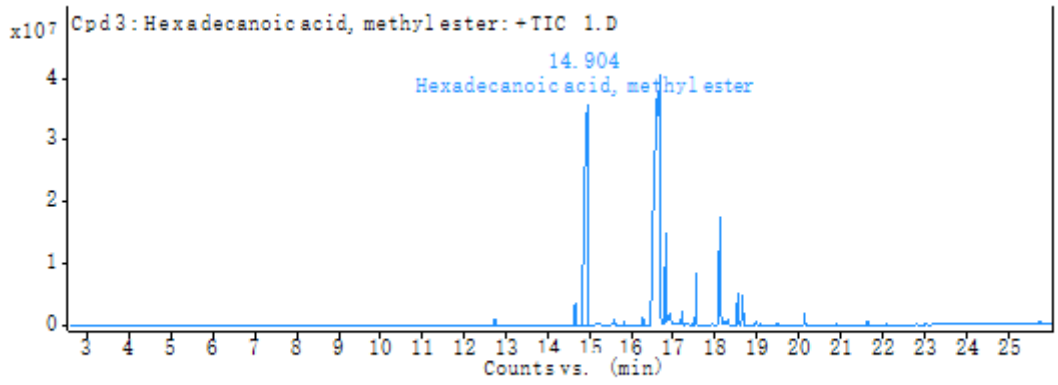

Fig.2-1 Chromatogram of chemical constituent for keep time (14.904min)

Table 3 Spectrum peak list (14.904min)

m/z	z	Abundance
41.1		562225.75
43.1	1	801154.58
55.1	1	881129.8
69.1	1	580568
74.1	1	4437047.22
87.1	1	3328363.52
143.1	1	1414397.39
227.2	1	1408828.82
239.2	1	1147764.41
270.2	1	1579199.62

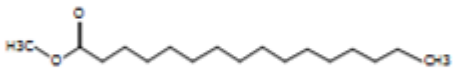

Fig.2-2 Compound structure of chemical constituent for keep time (14.904min)

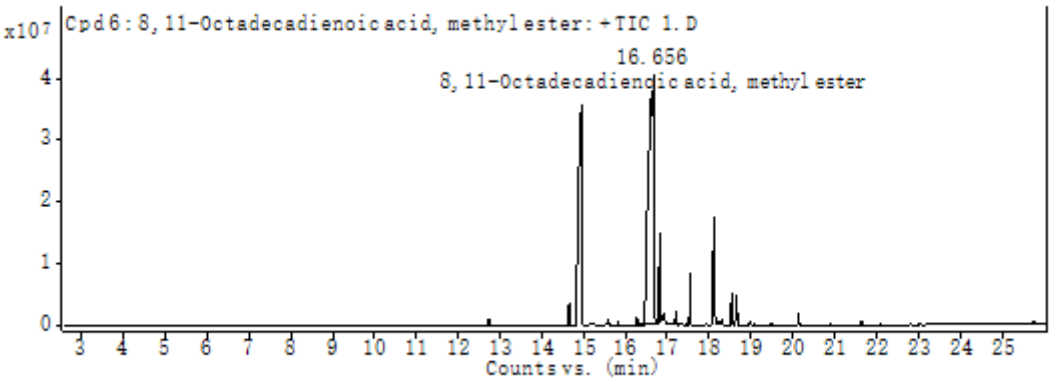

Fig. 3-1 Chromatogram of chemical constituent for keep time (16.656min)

Table 4 Spectrum peak list (16.656min)

m/z	z	Abundance
41.1	1	731846.59
55.1	1	1182681.41
67.1		1297575.38
69.1	1	853852.28
81.1		1368431.63
82.1		772121
95.1		1115022.75
96.1		942165.31
109.1		633604.94
294.2	1	683892.99

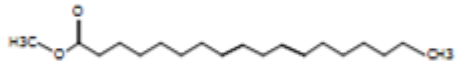

Fig. 3-2 Compound structure of chemical constituent for keep time (16.656min)

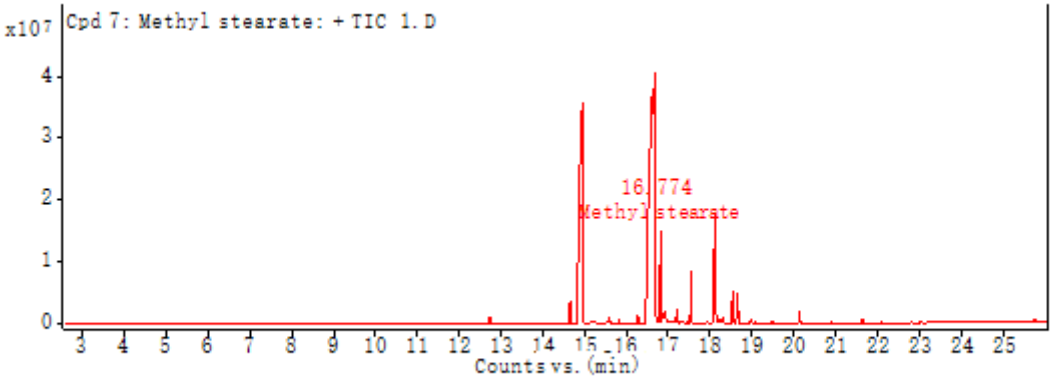

Fig. 4 -1 Chromatogram of chemical constituent for keep time (16.774nm)

Table 5 Spectrum peak list (16.774min)

m/z	z	Abundance
43.1	1	314969.38
55.1	1	335548.88
69.1	1	230203.88
74.1	1	1570023.75
87.1	1	1124663.88
143.1	1	472018.88
199.1	1	257344.25
255.2	1	485245.88
267.2	1	308738.63
298.2	1	677021.63

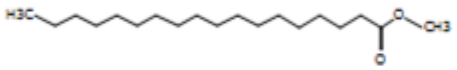

Fig. 4-2 Compound structure of chemical constituent for keep time (16.774min)

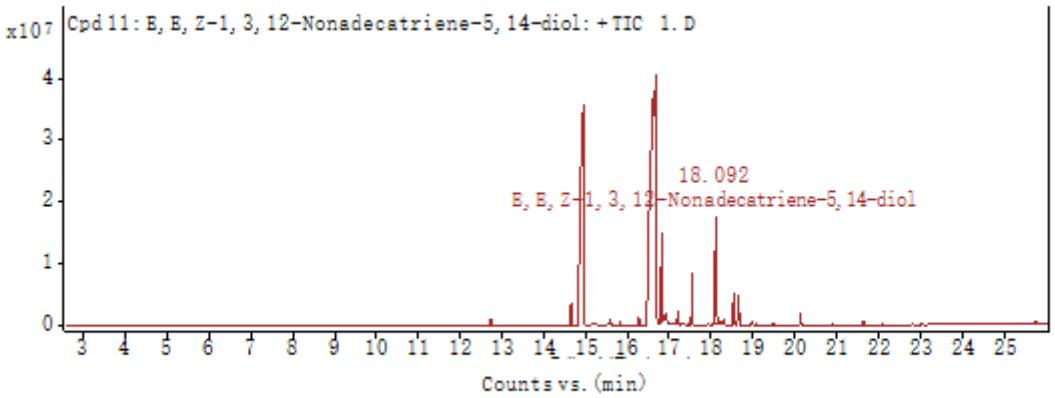

Fig. 5-1 Chromatogram of chemical constituent for keep time (18.092min)

Table 6 Spectrum peak list (18.092min)

m/z	z	Abundance
41.1	1	303592.35
55.1	1	483352.29
67.1		390939.63
68.1		271653.09
69.1	1	320673.54
81.1		473188.38
83.1		266377.47
95.1		343674.91
99.1		217911.27
147.1	1	237172.63

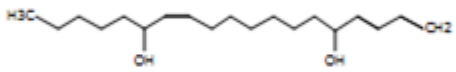

Fig. 5-2 Compound structure of chemical constituent for keep time (18.092min)

Linoleic acid content is 58.36%, Hexadecanoic acid content is 21.95%, Stearic acid content is3.7%, are more higher than other fatty acid contents .

3 Discuss

From the above experimental results, it can be seen that the main chemical constituents of Hibiscus Manihot seed fatty acids are unsaturated fatty acids. Studies have shown that unsaturated fatty acids are fatty acids that cannot be synthesized and are essential in the human body. They have the functions of relieving excess cholesterol in the blood, enhancing cell membrane permeability, preventing myocardial tissue and atherosclerosis [11-12]. How much of the body's intake of unsaturated fatty acids can also directly affect the synthesis of prostaglandin, has many effects on the body. Among them, the lack of linoleic acid causes cholesterol to bind to some saturated fatty acids and cause metabolic disorders. Hibiscus Manihot seed has high nutritional value and is a natural health food with great development potential.

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